THE DEVELOPMENT OF STROPHARIA AMBIGUA

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(WITH PLATES 124 AND 125, CONTAINING 12 FIGURES)

In the fall of 1911, the writer's attention was called to an agaric which is very conspicuous in fir woods in the vicinity of Seattle, Washington, during the fall and winter months. Specimens have been collected as late as January 17. For this work the very young stages were collected in the fall of 1912. As far as the writer is aware, no study of the development of the genus *Stropharia* has been published and there appears to be doubt about the taxonomy of this particular species.

The earlier literature on the development of the fruiting bodies of the Agaricaceae has been thoroughly reviewed by Atkinson (2), Allen (1), and Beer (3). In 1906, Atkinson found that in the early stages in the development of *Agaricus campestris* there was no differentiation, but a universal veil surrounded the homogeneous mass of hyphae. The first differentiation was the primordium of the hymenium in the form of a deeply stained ring a little above the center of the carpophore and lying some depth under the surface. The gill cavity forms below this hymenium, and the primordium of the pileus is distinguished from that of the stem and marginal veil. Next in the order of development the pileus becomes definitely outlined quite deeply under the surface by taking a deep stain.

In the same year, Allen (1) found the development of *Hypholoma* to be different from that of *Agaricus*. In *Hypholoma* the universal veil is present from the beginning, and the first differentiation is a small central area which stains deeply. This differentiates successively into the primordium of the pileus, hymenium, and upper portion of the stem. The gill cavity is formed internally after the formation of the primordium of the hymenium, and the lamellae are formed by the differential growth of the hyphae of the hymenial primordium.

Three years later, Fischer (4) published his work on the development of *Armillaria mucida,* in which he observes that the hymenial primordium has an endogenous origin, but that the primordium of the pileus precedes the appearance of that of the hymenium.

In 1911, Beer (3) followed with his notes on some species of Agaricaceae. In his work upon *Hypholoma fasciculare,* he practically confirmed what Allen (1) had already reported for *Hypholoma,* while in *Armillaria mellea* he found the sequence of the differentiation of parts and their development to correspond very closely with Atkinson's observations upon *Agaricus campestris.*

During November, 1912, specimens were collected on the campus of the University of Washington. No trouble was experienced in finding all stages from the youngest to the fully developed carpophores. The mycelium in the form of white, silky rhizomorphs was found just under the surface of the decayed organic matter on a moss-covered log of *Alnus oregana.* The rhizomorphs measure about 0.5–1 cm. in diameter. The pure white buttons were easily obtained from the surface of this substratum. Several mature carpophores aided much in locating the tiny button forms, which were readily identified by their viscid upper surfaces. Buttons 1–5 mm. in diameter, and portions of more mature carpophores were fixed in chromo-acetic acid and were carried through alcohol and xylol into paraffin. The sections were generally cut 6 μ thick. In staining, the best results were obtained with acid fuchsin and picric acid, to bring out the early differentiations. The safranin, gentian-violet, and orange combination was used to advantage in older stages. A large number of slides were made and the accompanying plates were photographed from slides chosen from these.

The early stage of the carpophore is an undifferentiated mass of interwoven hyphae which reaches a height of about 1.5 mm. At this time it is about 1 mm. in transverse diameter. Figure 1 shows the earliest stage. Over the surface of this primordial carpophore there is a layer of coarser, more loosely arranged hyphae. This is the universal veil. For the most part, the hyphae of the interior extend vertically from the base where the carpophore is connected with the rhizomorph. Sections of the latter

show it to be made up of a pseudo-parenchymous tissue. In the upper part of the carpophore the hyphae seem to spread and their course is generally parallel with the surface.

The first sign of internal differentiation of the previously homogenous tissue appears in a plane a little above the center of the primordium of the carpophore. Here in medial vertical section two darkly stained patches of descending hyphae appear (Fig. 2). These seem to be the regions of most active growth and are rich in protoplasm. In the remaining sections of the same carpophore these deeply stained areas can be traced as an annular area in the whole carpophore. This is the hymenial primordium. The hyphae of this region are very slender and pointed at first, but eventually they enlarge and become crowded, their lower ends forming an even surface. After this differentiation to form the primordium of the hymenium, an inverted cup-shaped layer of hyphae, rich in protoplasm appears. This extends upward from the outer edge of the hymenial primordium and over the top of the carpophore, remaining the same distance from the surface over the whole area. This forms the primordium of the pileus (Fig. 3). Simultaneously with the differentiation of the pileus the gill cavity is formed by the sagging of the neutral tissue below the hymenial primordium, due to the cessation of growth in that region. Figure 4 shows the primordium of the hymenium enlarged. The primordium of the hymenium increases in width at the outer edge, where the hyphae begin to grow downward and inward as if to form the incurved margin of the pileus. At this stage the partial veil can be distinguished as tissue of lighter stain, extending from the universal veil and the pileus margin to the outer surface of the upper portion of the stem, which is now clearly differentiated (Fig. 5).

Next, a differential growth takes place in the hymenial hyphae. Radial plates of these hyphae grow downward rapidly and form ridges, which are the first signs of the lamellae. As soon as these appear, the hyphae of the gills spread laterally, leaving a groove along the edge of the lamella. In the very earliest stages of the development of the lamellae they are differentiated into a lightly staining central region, and the heavily staining lateral regions made up of the tips of the hyphae. The vertical tangential sec-

tions (Figs. 6 and 7) show this feature. The central light region is the primordium of the trama of the lamella, while the heavily staining lateral regions are sections of the hymenium of the lamella. The broadening of the lamellae is brought about by the downward intercalary growth of fine, sharp-pointed hyphae in the trama. When their tips reach the groove at the edge of the lamella, they turn horizontally to form the hymenium. The hyphae of the hymenium are not large enough at this stage to stand out distinctly, but soon distinct, scattered, swollen hyphae stand out above the hymenium surface. These are cystidia. They are clavate, measure 20–22 μ long and 8–10 μ broad, and have large, deeply staining nuclei 4–6 μ in diameter.

As the lamellae grow in width, the trama becomes thicker because of the intercalary growth of new hyphae. But in a later stage, as shown by the sections of older lamellae (Fig. 8), the hyphae of the trama have increased in diameter causing the thickening of the trama in the upper part of the lamellae. At this stage, as Allen (1) also observed in *Hypholoma,* the cystidia appear greatly separated, indicating that some intercalary growth has taken place in the hymenium. A few observations have led to the belief that this hymenial growth is due to the branching of hyphae at the clamp connections in the subhymenium. The basidia are of the typical form and have four spores.

At an early stage, when the carpophores are about 4 mm. in diameter, the portion of the universal veil directly above the pileus dissolves into a viscid layer, and the cortical layer of the pileus secretes enough viscid substances to keep it coated through the rest of its development. In older specimens this viscid layer is about 0.1 mm. thick (Fig. 8). However, very small, pure-white patches of the universal veil may persist along the outer margin of the pileus until late stages. Figures 9 and 10 show this character and also the thick partial veil completely concealing the lamellae. In a still later stage this partial veil ruptures about midway between the stem and the margin of the pileus. It is thus left partially appendiculate to the margin of the pileus and partially as a distinct, white annulus which is striately lamellate on its upper surface (Figs. 10–12). In some specimens the

annulus is early evanescent, but in most it persists to old age. It is difficult to keep dried specimens with the annulus intact.

This species was reported as new by Peck (6) in 1898. The specimens from which the determination was made were sent by Dr. Lane from Portland, Oregon. Peck says, "The dried specimens have the general appearance of some species of *Stropharia,* but the appendiculate character of the veil and the *entire absence* of an annulus indicate that the species is a *Hypholoma.*" Then, in 1912, Murrill (5), in summing up the species of *Hypholoma* of the Pacific Coast, says of this one: "The species belongs naturally in *Stropharia,* but the large veil is *entirely* appendiculate and leaves *no* annulus." Figure 12 is a photograph of a specimen of my collection, No. 91, referred to by Murrill (5). The dried specimens of this collection which were sent to him for determination probably have no annulus intact.

In the light of the present investigation there are two lines of differentiation between this species and *Hypholoma.*

First, in the early states of *Hypholoma,* as worked out morphologically by Allen (1) and later verified by Beer (3), the differentiation of the parts does not correspond to that of this species. In *Hypholoma* the differentiation of the pileus preceded the other parts. Beer (3) also says that in *Clitocybe laccata* "the first differentiation of the carpophore primordium consists in the demarcation of the pileus." In *Stropharia ambigua* the first differentiation is the appearance of the primordium of the hymenium. Atkinson (2) found this true in *Agaricus campestris,* and Beer (3) observed the same order of development in *Armillaria mellea.* Thus, according to our present knowledge of the development of the carpophores of the Agaricaceae, with one exception the annulate forms develop the hymenial primordium first, while other forms develop the primordium of the pileus first. Fischer's work (4) on an annulate form may show an exception; but it seems to the writer that according to Fischer's findings the differentiation of the hymenium brings about the differentiation of the pileus, and Beer (3) suggests that the differentiation of the pileus and hymenium in this case is possibly simultaneous. Further investigation on these two types has been started by the writer.

Second, there is an annulus present in this species. This feature has been noticed with interest since specimen No. 91 was determined (5). Students in a course in Fungi at the University of Washington have invariably traced it to the genus *Stropharia*. It is true that a part of the veil is characteristically appendiculate but the greater part forms a pendulous annulus, which is thick, membranaceous, and pure-white, but for the purplish-brown edges of the striate lamellae on the upper surface. The annulus is cone-shaped, has a fimbriate margin, and is fixed.

Since it is evident from these two standpoints that this plant has been taxonomically misplaced, the new combination **Stropharia ambigua** (*Peck*) is proposed.

The lamellated upper surface of the annulus brings *S. ambigua* into close relationship with the little *S. bilamellata* Peck (7). However, *S. ambigua* is much larger and leaves a portion of the veil appendiculate.

The writer is under obligations to Dr. J. W. Hotson for helpful suggestions in this work.

SUMMARY

1. The species in question does not develop like *Hypholoma*, but like the annulate forms.

2. In its young stages it has an annulus which is sometimes evanescent.

3. Therefore the new combination, **Stropharia ambigua** (Peck), is proposed.

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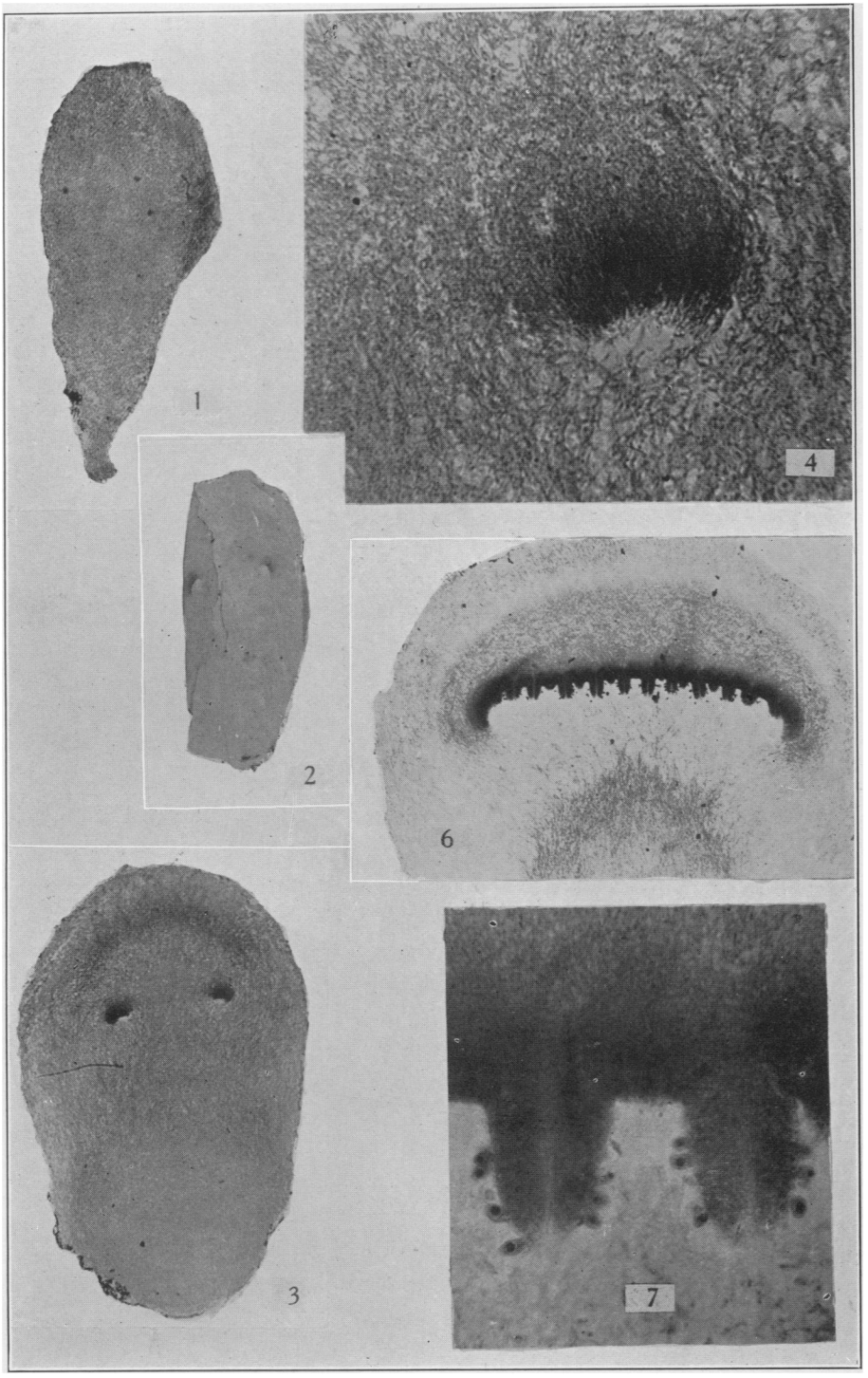
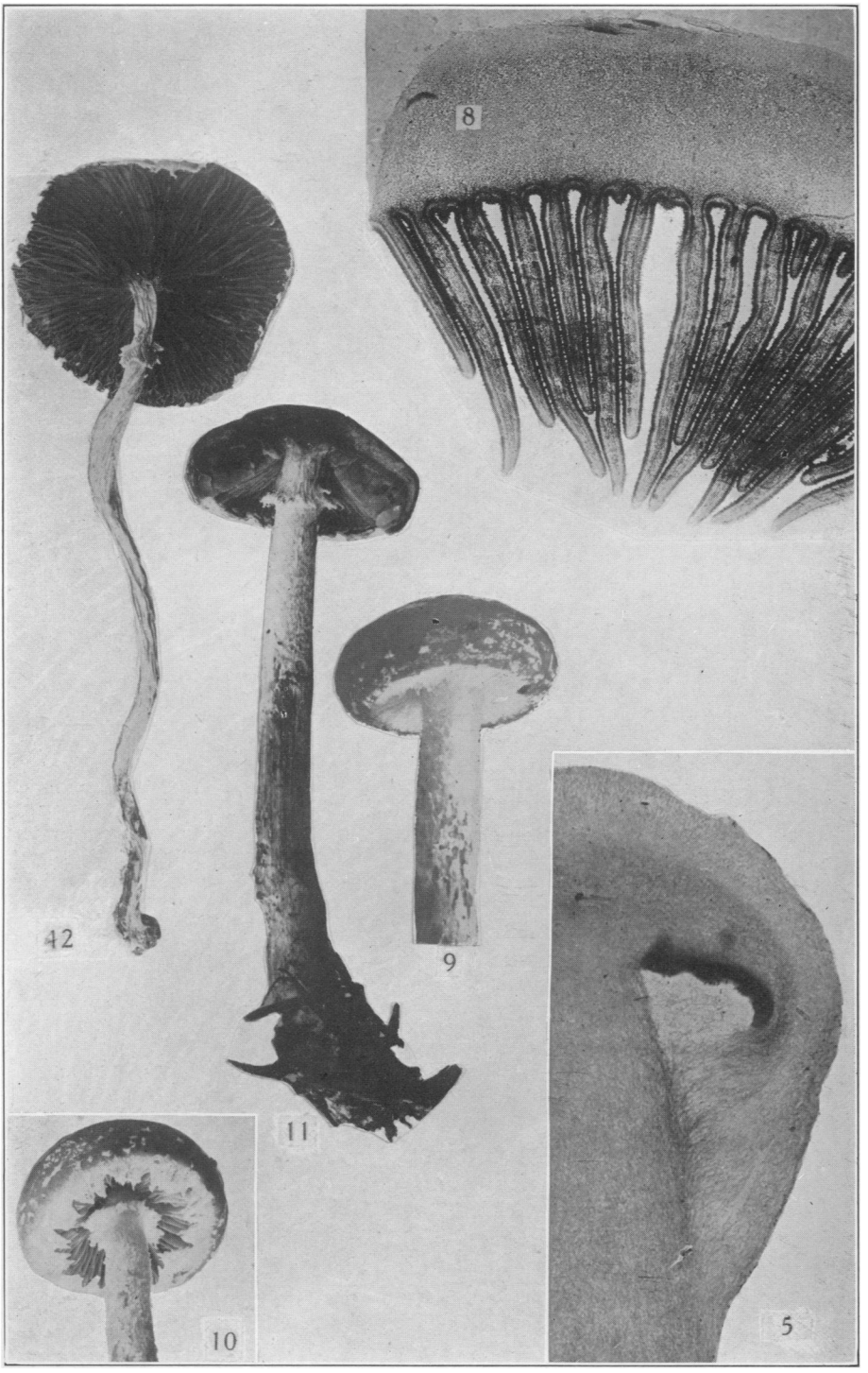
LITERATURE CITED

1. **Allen, Caroline L.** The Development of Some Species of *Hypholoma*. Ann. Myc. 4: 387–394. 1906.
2. **Atkinson, Geo. F.** The Development of *Agaricus campestris*. Bot. Gaz. 42: 241–264. 1906.
3. **Beer, Rudolf.** Notes on the Development of the Carpophore of Some Agaricaceae. Ann. Bot. 25: 683–689. 1911.
4. **Fischer, C. C. E.** On the Development of the Fructification of *Armillaria mucida* (Schrad.). Ann. Bot. 23: 503–507. 1909.
5. **Murill, W. A.** Agaricaceae of the Pacific Coast. Mycologia 4: 304–305. 1912.



STROPHARIA AMBIGUA (PECK) ZELLER



STROPHARIA AMBIGUA (PECK) ZELLER

6. **Peck, Chas. H.** New Species of Fungi. Bull. Torrey Club **25**: 325–326. 1898.

7. **Peck, Chas. H.** New Species of Fungi. Bull. Torrey Club **22**: 204. 1895.

DESCRIPTION OF PLATES CXXIV AND CXXV

Photomicrographs by Homer O. Blair and photographs by the author.

Fig. 1. Young carpophore undifferentiated; × 30.

Fig. 2. Young carpophore with hymenial primordium developed, but no evidence of the primordium of the pileus; × 30.

Fig. 3. Young carpophore showing the hymenial primordium farther developed than in Fig. 2 and the appearance of the primordium of the pileus; × 30.

Fig. 4. From same young carpophore as Fig. 3; the primordium of the hymenium showing the beginning of the gill cavity; × 300.

Fig. 5. Part of young carpophore showing the gill cavity; the primordium of the hymenium continues to develop as the margin of the pileus continues to grow; young lamellae in longitudinal section; partial veil of loose filaments; and cortex of the stem; × 30.

Fig. 6. Tangential section of young carpophore showing young lamellae in cross section; the lightly staining central portion is the trama; cystidia; × 30.

Fig. 7. Greater magnification of the same lamellae as shown in Fig. 6, showing the trama, hymenium and cystidia with large spherical nuclei; × 300.

Fig. 8. Tangential section of more mature pileus and lamellae; the trama of the pileus; superficial viscid layer; trama, subhymenium and hymenium of the lamellae; × 60.

Fig. 9. Pileus of carpophore showing small flocculent patches of the universal veil and the partial veil completely covering the lamellae. Nat. size.

Fig. 10. Carpophore showing partial veil ruptured leaving annulus and partly appendiculate. Nat. size.

Fig. 11. Same object as Fig. 10 with part of pileus cut away to show annulus. Nat. size.

Fig. 12. Dried specimen (Zeller, No. 91) showing annulus. Nat. size.